DETERIORATION OF SPINAL REFLEX IN QUAILS ORALLY INGESTING Clioquinol

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Ataxia occurred in quails on long-term oral ingestion of clioquinol (CQ). Crossed extensor reflexes and spinal reflex potentials in the normal and the treated quails were recorded. The incidence of these reflexes was reduced in quails given CQ. As long as the spinal reflexes were detected, there were no prominent differences in the profiles of the reflex potentials between the normal groups and those with ataxia. Augmentation of the reflex potentials by strychnine in the treated group was more extensive than that in the normal group. The mechanisms of action by which CQ induces ataxia are discussed.

**Keywords** — clioquinol; spinal reflex; crossed extensor reflex; quail; ataxia

INTRODUCTION

Long-term oral administration of clioquinol (CQ) induces neurological symptoms and patho-logical changes similar to events seen in cases of subacute myelopathy in dogs, cats, monkeys, chicks and quails. Recently, Kotaki et al. reported that long-term intraperitoneal administration of CQ induced ataxia in rats. We reported that in beagles given CQ orally, motor paralysis of the hindlimbs developed and the response with long latency of the spinal reflex potential disappeared in the treated animals. We now report that in the majority of quails repeatedly given oral CQ, ataxia occurred. Crossed extensor reflexes and spinal reflex potentials were studied both in the normal and the treated quails.

MATERIALS AND METHODS

**Drug Administration** — Seventy-five cross-bred male quails were grouped 7, of A—G and were given CQ orally by gavage once daily from 8 d after hatching, except for Sundays and National holidays. The schedule of CQ administration is shown in Table I. Twenty-four to 38 d after the start of administration, crossed extensor reflexes of quails in groups A, B, and C, and spinal reflex potentials of group D, E, F and G were recorded.

**Crossed Extensor Reflexes** — The procedure and recordings were the same as in our previous report. Under anesthesia with a-chloralose (50 mg/kg, i.p.), quails were fixed in a dorsal position. After insertion of a cannula into the trachea, the superficial bular nerve of the left leg was exposed and stimulated (0.2 Hz, duration 1 ms, Nihonkoden MSE-40) to induce the contralateral extensor reflex. The threshold voltage was assessed following a gradual acceleration of the voltage. Movements of the right leg following supramaximal stimulation were recorded by means of an isotonic transducer (Nihonkoden, TD-111s).

**Spinal Reflex Potentials** — Quails were anesthetized with a-chloralose (50 mg/kg, i.p.) and immobilized with d-tubocurarine chloride (2 mg/kg, i.p.). Under artificial respiration, they were fixed in a dorsal position, and immersed in

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TABLE I. Dosing Schedule of Clioquinol in Studies of the Crossed Extensor Reflex and the Spinal Reflex Potential in Quails

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
<th>Group G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 2</td>
<td>Vehicle</td>
<td>30</td>
<td>100</td>
<td>Vehicle</td>
<td>100</td>
<td>Vehicle</td>
<td>100</td>
</tr>
<tr>
<td>3 – 4</td>
<td>Vehicle</td>
<td>100</td>
<td>300</td>
<td>Vehicle</td>
<td>300</td>
<td>Vehicle</td>
<td>300</td>
</tr>
<tr>
<td>5 – 6</td>
<td>Vehicle</td>
<td>300</td>
<td>1000</td>
<td>Vehicle</td>
<td>1000</td>
<td>Vehicle</td>
<td>1000</td>
</tr>
<tr>
<td>7 – 8</td>
<td>Vehicle</td>
<td>300</td>
<td>1000</td>
<td>Vehicle</td>
<td>2000</td>
<td>Vehicle</td>
<td>2000</td>
</tr>
<tr>
<td>9 –</td>
<td>Vehicle</td>
<td>300</td>
<td>1000</td>
<td>Vehicle</td>
<td>1000</td>
<td></td>
<td>a)</td>
</tr>
<tr>
<td>&gt;24</td>
<td>Crossed extensor reflex</td>
<td></td>
<td>Spinal reflex potential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Not administered.

Liquid paraffin kept at 36 – 37 °C. After isolation of both sides of tibial and peroneal nerves, the left peroneal nerve was stimulated (frequency 0.2 Hz, duration 0.05 ms, 5 V, Nihonkohden MSE-40), and peroneal nerve-peroneal nerve reflex potentials (P-PR) and peroneal nerve-tibial nerve reflex potentials (P-TR) were evoked in the right peroneal and tibial nerves, respectively. These reflex potentials were recorded by memory oscilloscope (Nihonkohden, VC-10A) and recticorder (Nihonkohden, RJG-4122). Thirty minutes after the start of stimulation, strychnine nitrate (1 mg/kg, i.p.) was administered, and 15 min after the injection spinal transaction was performed.

Chemicals — Drugs used were cliquinol (CQ, Sigma), strychnine nitrate (Wako), α-chloralose (Tokyo-kasei) and d-tubocurarine chloride (Tokyo-kasei). CQ was suspended in 1% sodium carboxymethyl cellulose (CMC) solution and other drugs were dissolved in physiological saline.

RESULTS

Behavioral Changes

There were no differences in body weight changes among the 7 groups of quails, except for group G (Table I) in which increments of body weight were suppressed at days 12 and 13. No quails in groups A, D and F of the control and group B given the low dose developed motor disturbance. Behavioral changes of quails of groups C, E, and G, who received CQ, are shown in Figs. 1 and 2. In groups C, E, and G, the ataxia began to develop from day 8 and then increased. In all the surviving quails ataxia developed. The first symptom was difficulty in walking and the animals rested frequently (light ataxia). In the advanced stage, the animals fell down as they could not retain their balance when forced to move by pushing them from the side (heavy ataxia). In some quails, tonus developed in the hindlimb. Even under these circumstances, the

animal No. | Days after start of administration
0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45
---|---|---|---|---|---|---|---|---|---|---
2 |   |   |   |   |   |   |   |   |   |   
6 |   |   |   |   |   |   |   |   |   |   
7 |   |   |   |   |   |   |   |   |   |   
11 |   |   |   |   |   |   |   |   |   |   
12 |   |   |   |   |   |   |   |   |   |   
14 |   |   |   |   |   |   |   |   |   |   
20 |   |   |   |   |   |   |   |   |   |   
21 |   |   |   |   |   |   |   |   |   |   
25 |   |   |   |   |   |   |   |   |   |   
27 |   |   |   |   |   |   |   |   |   |   

FIG. 1. Behavioral Changes of Quails Given Clioquinol

Clioquinol was administered continuously except for Sundays and National holidays. ○, normal; ○, light ataxia: Difficulty in Standing; ●, heavy ataxia: Falling down when forced to move; ♦, tonus in hindlimb.
quails had a good appetite. In groups E and G, heavy ataxia occurred in the majority of quails at 10 d after the start of administration. However, a few quails died around this time. Quails in group E were continuously given a daily dose of 1000 mg/kg, and in the quails of group G, administration of CQ was stopped. In the latter group, there was a trend toward recovery from ataxia in 1/2 quails, although, only one quail recovered completely.

Crossed-Extensor Reflexes

Crossed-extensor reflex was present in all quails in groups A and B. However, the reflex of group C was negligible in 5/9 quails and statistically different from group A, in which all of 10 quails developed the reflexes (Fisher's exact probability method \( p<0.05 \)) (Fig. 3). In quails whose reflexes were detected, the mean thresholds of stimulus and their standard errors were 0.69 ± 0.21, 2.23 ± 1.54 and 3.03 ± 1.34 V in groups A, B, and C, respectively. The threshold voltage of stimulus tended to increase with CQ administration; however, the difference between controls and CQ treated groups was not significant.

Spinal Reflex Potentials

In quails in groups D and F (control groups), evoked P-PR and P-TR were observed with 30 to 130 \( \mu \text{V} \) of the maximum amplitude, 9 to 10 ms of minimum latency, and 3 to 15 ms of duration. There were no differences in the spinal reflex potentials between the CQ administered and control groups. Maximum amplitude and duration of these reflex potentials increased after the administration of strychnine (Fig. 4). Data of spinal reflex potentials of the quails of groups D, E, F, and G are shown in Tables II and III.

After the administration of strychnine, the reflex potentials were detected in the majority of quails in groups D, F, and G. However, in group E, the reflex potentials were not detectable in half the number of quails. In the animals whose reflex potentials were detected, minimum latency of P-TR of the quails of group E was longer than that of group D. After strychnine ad-

![FIG. 2. Behavioral Changes of Quails Given Clioquinol](image)

Clioquinol was administered orally, except for Sundays in group E, and group G was given clioquinol for the first 8 days only.

○, normal; ●, light ataxia: Difficulty in standing; ●●, heavy ataxia: Falling down when forced to move; ♦, tonus in hindlimb.

![FIG. 3. Crossed Extensor Reflexes in Quails Given Clioquinol](image)
ministration, the duration of P-PR and P-TR of group E was longer than that of group D, and the minimum latency of these reflex potentials tended to be longer. The latency of P-TR of group G was longer than that of group F. The duration of P-PR and P-TR of group G tended to be longer. The response with long latency (30 ms) of P-PR and P-TR of group E disappeared after spinal transection (Fig. 4).

DISCUSSION

We found that long-term oral administration of CQ induced ataxia in quails, the incidences of crossed extensor reflexes and spinal reflex potentials were reduced by CQ and augmentation of the spinal reflex potentials by strychnine in the CQ administered group was greater than that in the controls.

Most of the quails did not die when given the final dose of 1000 or 2000 mg/kg/d of CQ, and retained the ataxia. Others reported that quails died within a few days after ataxia produced by long-term oral administration of CQ.\(^5\) The ataxia did not disappear after the withdrawal of CQ administration, thereby suggesting that the ataxia is caused by a chronic injury of the nervous system.

Low incidences of the crossed extensor reflexes and spinal reflex potentials, and trends in increment of threshold voltage to induce crossed-extensor reflex suggest that one of the sites of injury is the sciatic nerve. This possibility is supported by the following: In histological examination of sciatic nerves of rabbits with CQ-induced ataxia, Igata et al.\(^9\) observed swelling of axons and decomposition of the medullary sheath. Using radioisotope labelled CQ, Takasu et al.\(^10\) reported that high levels of CQ were incorporated into the sciatic nerve in beagles.

Strychnine potentiates the spinal reflex potentials by inhibition of the inhibitory neurons.\(^11\) Although there were no prominent differences in the spinal reflex potentials between CQ administered and control groups, augmentation of these reflex potentials by strychnine in the former group was greater than that in latter group. Duration of the reflex potentials was prolonged, and the minimum latency tended to be longer. This suggests that inhibitory neurons are injured by CQ administration and are readily inhibited by strychnine. Data to support this proposal have not been obtained.

In some quails given CQ, the reflex potentials with a long latency (30 ms) were observed. As these reflex potentials disappeared after spinal transection, they may be spino-bulbo-spinal ones. However, in our previous paper,\(^7\) reflexes with a long latency disappeared following CQ administration to beagles. The discrepancy might be related to species differences, to the anesthesia, or to the method of experiment. In the present experiment, the tibial nerve was stimulated directly and evoked potential was recorded, whereas in the previous experiment the superficial fibular nerve was stimulated indirectly and the evoked potential was assessed by electromyography.

Augmentation of the reflex potentials, i.e. extension of the minimum latency was also observed in quails in which the ataxia had not disappeared after the withdrawal of CQ. The low incidence of the spinal reflex potential was

![Typical Spinal Reflex Potentials in Quails Given Clioquinol Orally](image-url)
### TABLE II. **Spinal Reflex Potentials in Quails Given Clioquinol (Group E)**

<table>
<thead>
<tr>
<th></th>
<th>Peroneal-peroneal-reflexes</th>
<th>Peroneal-tibial-reflexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude (μV)</td>
<td>Latency (ms)</td>
</tr>
<tr>
<td><strong>Control (Group D)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>46.1 ± 8.8 (9/10)</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>Strychnine (1 mg/kg, i.p.)</td>
<td>435.0 ± 75.0 (10/10)</td>
<td>11.2 ± 1.0</td>
</tr>
<tr>
<td>Spinal transection</td>
<td>356.7 ± 75.2 (10/10)</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td><strong>Treated (Group E)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>75.0 ± 50.8 (5/10)</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>Strychnine (1 mg/kg, i.p.)</td>
<td>411.0 ± 90.4 (6/10)</td>
<td>15.2 ± 1.9</td>
</tr>
<tr>
<td>Spinal transection</td>
<td>530.4 ± 106.4 (5/10)</td>
<td>14.6 ± 2.3</td>
</tr>
</tbody>
</table>

*The data are expressed as mean ± S.E. Figures in parentheses indicate the incidence of the reflexes. a) p<0.05, b) p<0.01 as compared with control group using the t-test. c) <0.05 as compared with control group using Fisher’s exact probability method.*

### TABLE III. **Spinal Reflex Potentials in Quails Given Clioquinol (Group G) for 8 Days Only**

<table>
<thead>
<tr>
<th></th>
<th>Peroneal-peroneal-reflexes</th>
<th>Peroneal-tibial-reflexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude (μV)</td>
<td>Latency (ms)</td>
</tr>
<tr>
<td><strong>Control (Group F)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>128.9 ± 51.5 (6/10)</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>Strychnine (1 mg/kg, i.p.)</td>
<td>489.1 ± 144.6 (10/10)</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>Spinal transection</td>
<td>452.1 ± 178.8 (10/10)</td>
<td>10.4 ± 0.3</td>
</tr>
<tr>
<td><strong>Treated (Group G)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>24.2 ± 8.5 (3/7)</td>
<td>9.9 ± 1.5</td>
</tr>
<tr>
<td>Strychnine (1 mg/kg, i.p.)</td>
<td>147.3 ± 53.7 (7/7)</td>
<td>15.7 ± 2.4</td>
</tr>
<tr>
<td>Spinal transection</td>
<td>121.4 ± 58.6 (6/7)</td>
<td>12.6 ± 0.8</td>
</tr>
</tbody>
</table>

*The data are expressed as mean ± S.E. Figures in parentheses indicate the incidence of the reflexes. a) p<0.01 as compared with control group using the t-test.*
recovered following withdrawal of the CQ. Thus, there may be relationships between the development of ataxia and augmentation of the reflex potentials.

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REFERENCES